# A SUGGESTION AS TO THE EFFECT OF THYROID GLAND SUBSTANCES ON PROTOPLASM IN GENERAL.[1]

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Of the numerous angles from which the physiology of the thyroid gland has been approached, that of its influence during embryological development has been frequently studied. It is quite unnecessary for the purposes of the present paper to review the large literature of the subject; bibliographies are abundant. Briefly, one may recall that the effect of administering thyroid material to children is well known; Gudernatsch ('12), followed by many others, showed the precocious differentiation which follows the feeding of thyroid gland to tadpoles of Amphibia; Kunkel ('18) noted the response of flesh-fly larvæ to thyroid feeding; its influence on other phyla of animals has been investigated, even the Protozoa having been shown to react with an accelerated rate of metabolism, many structural modifications, a faster rate of reproduction, etc., to a thyroid diet.

In all instances of experimental research the reacting substance is, of course, protoplasm. Its condition, highly organized into the various specialized tissues, studied, experimented upon, and interpreted *en masse*—these facts, and our habits of thought, too often lead us into temporary forgetfulness of *protoplasm* as the fundamental and only vital substance in the material being studied.

As intimated above, it would seem that so many animals from such distinct levels of organization have shown clear responses to absorbed thyroid materials that we may easily conclude that something in thyroid composition affects and modifies animal protoplasm perhaps whenever and wherever it occurs. Correlated with this semihypothetical conclusion, one reflects that

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there is another large world of protoplasm identified with the plant kingdom; and the question arises, "Is *all* protoplasm enough alike so that that of plant associations, although of course never normally exposed to thyroid influences, is, never-the-less, susceptible to them?" If so, then the interest which attaches to the physiological effect of this endocrine substance is much increased; if not, then one of the differences in their characteristics, which perhaps was instituted when animal and plant protoplasms began their long evolutionary divergence, is made roughly apparent.

A further question which arises is: "Are animal and plant protoplasms sufficiently alike so that, if both are susceptible to thyroid materials, they both react in the same manner?" That they apparently do to some infections has been fascinatingly brought out by the investigations of Smith ('20) in his scholarly studies into the nature of crown gall in plants, and its close similarity, etiologically, to human cancer. Very numerous instances of similar or comparable responses of plants and animals to the same physical stimuli could be cited.

The fact which has been taken as a point of departure in the present study is the use of thyroid tissues or extracts in hastening differentiation of vertebrate larval cell-masses into adult-like organs, and the provoking of a general precocious metamorphosis. The query, then, is: "Can thyroid gland materials cause precocious differentiation of unspecialized plant tissues?"

## Experimental Methods and Results.

The observations here described were made entirely on roots growing from bulbs of *Narcissus*. Naturally, selection of a root must be made with reference to the special tissue structure it may exhibit. In some forms there is a clear-cut separation between the root-cap and its adjacent tissues; in such cases the cap may be cleanly removed from the adjoining tissues, there being no derivational dependence between them. In other roots the cap-cells are continuous with those of the root proper, a "common initial zone" existing between them, from which zone cells bud off into both adjoining areas. It is to this second type, "Type 5" of Haberlandt ('14), that *Narcissus* belongs; as a convenient reminder of the circumstances, a plan of its root tissue arrangement is shown in a diagram of its longitudinal section in Fig. 1. Neither the "common initial zone" nor the root-cap shows any distinct differentiation; they form an essentially parenchymatous mass. In a proximal direction, however, differentiation is soon met, *i.e.*, the cylindrical periblem, and the centrally placed plerome mass, also a cylinder.

The real question, then, which is being applied to the *Narcissus* root is: "Do the specialized tissues of the root proper extend down farther into the tip in such roots as are grown in nutritive media containing thyroid constituents than they do in roots grown in nutrient media alone?"

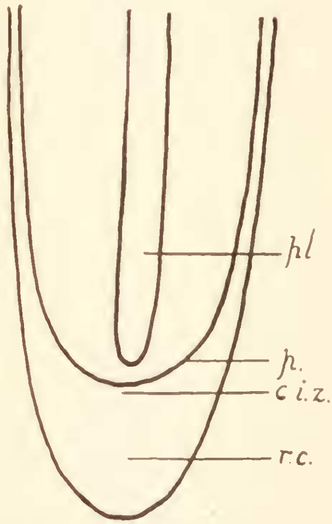


FIG. 1. Showing diagrammatically the arrangement of tissues in the root-tip of *Narcissus;* longitudinal section. *c.i.z.*, common initial zone; *p.*, periblem; *pl.*, plerome; *r.c.*, root cap.

Several dozens of *Narcissus* bulbs were rooted in bottles containing 120 cc. of Pfeffer's nutrient solution to which had been added gland substance (Parke Davis and Co.'s dry thyroid tablets) in these amounts: 2.5. grains, 5 grains, 7.5 grains, and 10 grains, respectively. It is well known, of course, that bulbs differ much in their "strength," and that in consequence of variation in vitality their root growth is not uniform in rate, length and other features; but there is an easily observable average which characterizes the normal or any experimental line. So far as bulk effect of thyroid substances on root growth in *Narcissus* goes, the result is the same as in *Allium*, reported by the writer in '19; in general, when the control bulbs showed a root length of 60 mm., the thyroid-"fed" line showed a length of 25 mm. These roots were then cut, fixed in aceto-alcohol, stained *in toto* in Delafield's hæmatoxylin, and embedded. Measurements of any region could be most accurately determined by a study of serial cross-sections; these were made 15 micra in thickness.

By count of sections, two measurements were made: (a) the interval from the tip of the root cap to the beginning of the periblem zone; (b) the interval from the tip of the root cap to the central plerome cylinder. To illustrate the uniformity of the result of such measurements, the following table is given, compiled from a series of thirty roots taken at random from among hundreds:

Table I.

Measurements Computed from Sections, 15 Micra in Thickness, of *Narcissus* Roots Growing in 120 cc. Pfeffer's Solution to which had been Added the Equivalent of 10 Grains of Thyroid Gland.

| | Micra from Tip of Root to Periblem. | Micra from Tip of Root to Plerome. |
|---|---|---|
| 1 | 360 | 405 |
| 2 | 360 | 420 |
| 3 | 375 | 420 |
| 4 | 345 | 405 |
| 5 | 360 | 420 |
| 6 | 330 | 405 |
| 7 | 345 | 405 |
| 8 | 360 | 435 |
| 9 | 405 | 465 |
| 10 | 390 | 480 |
| 11 | 300 | 360 |
| 12 | 330 | 390 |
| 13 | 420 | 495 |
| 14 | 390 | 435 |
| 15 | 390 | 450 |
| 16 | 330 | 400 |
| 17 | 405 | 465 |
| 18 | 360 | 405 |
| 19 | 330 | 390 |
| 20 | 405 | 450 |
| 21 | 330 | 390 |
| 22 | 375 | 420 |
| 23 | 375 | 420 |
| 24 | 390 | 465 |
| 25 | 360 | 405 |
| 26 | 390 | 465 |
| 27 | 360 | 420 |
| 28 | 390 | 465 |
| 29 | 390 | 465 |
| 30 | 300 | 390 |

In the familiar experiments with frog tadpoles, the thyroid-fed line and the control line are, of course, taken from the same batch of eggs, thus being of the same nature and the same age. Similarly, in this case, the bulbs were sprouted in the same nutrient medium, for the same length of time, the sole difference between the experimental and the control bulbs being that the former grew with a given amount of thyroid material added to the solution.

For comparison with the above table, measurements similarly made of the control line are here added:

### Table II.

Measurements Computed from Sections, 15 Micra in Thickness, of *Narcissus* Roots Growing in Pfeffer's Solution Alone.

| | Micra from Tip of Root to Periblem. | Micra from Tip of Root to Plerome. |
|---|---|---|
| 1 | 450 | 555 |
| 2 | 465 | 550 |
| 3 | 540 | 630 |
| 4 | 480 | 540 |
| 5 | 420 | 480 |
| 6 | 450 | 495 |
| 7 | 480 | 555 |
| 8 | 450 | 525 |
| 9 | 465 | 540 |
| 10 | 480 | 540 |
| 11 | 465 | 525 |
| 12 | 480 | 540 |
| 13 | 435 | 495 |
| 14 | 465 | 510 |
| 15 | 420 | 495 |
| 16 | 435 | 495 |
| 17 | 450 | 510 |
| 18 | 495 | 555 |
| 19 | 450 | 510 |
| 20 | 435 | 495 |
| 21 | 495 | 570 |
| 22 | 420 | 465 |
| 23 | 495 | 570 |
| 24 | 480 | 540 |
| 25 | 495 | 570 |
| 26 | 495 | 570 |
| 27 | 495 | 555 |
| 28 | 405 | 480 |
| 29 | 450 | 525 |
| 30 | 495 | 570 |

It seems perfectly clear that the differentiated tissue extends nearer to the tip of the root in the thyroid treated line than it does in the roots of the control line: or, in tangible figures, it extends 20 per cent. nearer the tip.

## Discussion

The profound and almost semi-mysterious control of metabolism by thyroid constituents, expressing itself mainly in the phenomena of growth, has attracted many workers to an examination of very varied phases of its possible influence. The present study is but one of this sort; and certain aspects of the facts noted may well receive further comment.

As said above, the main point in mind has been to discover whether thyroid components, if absorbed by elementary rapidly growing plant tissues, will cause in them an accelerated differentiation comparable with what has been repeatedly described as a distinct effect of it when used as a food by, grafted into, or introduced as an extract into Amphibian larvæ. A primary awkwardness immediately presents itself in such an attempt, for the plant root here employed shows such limited specialization as compared with the complexity of an incipient animal organ such as an appendage. The periblem and plerome tissues are the only real differentiations from the parenchyma in a young root, and they show little character in their apical extremities, their identification being essentially dependent on position only. They are, however, *bona fide* differentiations, as truly as muscle, bone, or nerve tissue.

Furthermore, it would be folly to claim that the experimental results here reported are unique to, specific for thyroid "feeding"; there may be dozens of organic and inorganic compounds, so far as the writer knows, which could produce similar or even greater effects of the sort noted than do thyroid components. No attempt has been made to discover or compile such a list. Potassium biniodide has often been employed to offset the effect of thyroid deficiency in children. This fact, however, does not at all lessen one's interest in thyroid substances and their normal physiological values throughout the life-time of the possessing organism.

It may also seem that the interpretation given the experimental results of this study, as indicating precocious specialization, is arbitrary as against other possible judgments. For example, does not a long root of *Narcissus* normally possess a root-cap thicker than that of a short root? To test this point, a series of roots grown in nutrient solution alone were cut when at the same or less length than those of the thyroid "fed" line, the question of relative age being disregarded. On making measurements of these it was found that the short normal root shows an extent of root-cap equal to or even in excess of that of a longer normal root. The question here at issue can be most easily met by offering the following table of averages of root-cap length (tip to periblem and plerome) of thirty roots each, taken from long-normal (control), short-normal, and thyroid "fed" groups:

| | Normal of Same Age as "Thyroid-fed." | Normal of Same or Less Length than "Thyroid-fed." | "Thyroid-fed." |
|---|---|---|---|
| Length of root up to beginning of periblem | 463 micra | 513 micra | 372 micra |
| Length of root up to beginning of plerome | 520.5 " | 576 " | 412.5 " |

It is thus plainly apparent that the cap of the experimental line was more deeply invaded by the specialized tissue than were either of the check roots with which there was any reason for comparing it.

One also naturally raises the question whether the results noted, instead of being those of accelerated differentiation, could not as well be described as simple abbreviation or inhibition of root-cap growth. It is possible that such an interpretation would be perfectly valid; at the same time, one should reflect that the effect is one, not on the root-cap alone, but on the entire root. While the foregoing measurements give information as to only one feature, *i.e.*, length of root-cap, other specifications are also modified by the treatment: *e.g.*, its entire length is less than that of the normal; its average diameter is greater at nearly every part of its growth; it shows tendencies to localized swellings and crookedness. Measurements of these other features of the thyroid "fed" line, however, it is impossible to make in any satisfactory manner; yet, they may all indicate precociousness. Assuredly, one reflects that hastened specialization of tissues in amphibian larvæ, in dipteran larvæ, in protozoa, were all accompanied by under-size; and Gudernatsch's ('15) experiments with a mammal showed the same diminished size in his thyroid fed line.

The further question whether all protoplasm reacts, and reacts similarly to thyroid components can hardly be answered, even tentatively, while the amount of study of plant responses is so small. Also, one can feel only the most limited concern whether any special plant tissue is amenable to such hormones or not; but the more general inquiry as to the qualities of protoplasm as a whole makes a real appeal. So far as the substance of this group of observations goes, the evidence would seem to suggest an affirmative answer.

## Summary.

1. Roots growing from bulbs of *Narcissus* into Pfeffer's nutrient solution to which has been added certain amounts of thyroid gland substance show a growth which is markedly abbreviated as compared with that of the control lines. This result is wholly like that when *Allium* is used.

2. Measurements directed to find out the internal conditions correlated with shorter growth indicate that differentiation of the special root tissues extends nearer to the tip of the root in thyroid "fed" lines than it does in the controls.

3. Assuming that the tissues in freshly growing plant roots can be compared to those in larval animals (Amphibia)—both originate from essentially unspecialized masses, although the plant never reaches the complexity of the animal—it would seem that thyroid substances cause precocious differentiation in both.

4. The suggestion is offered that animal and plant protoplasm are sufficiently alike in their general physiology so that they respond similarly to thyroid substances; or, expressed conversely, thyroid substances are influential in essentially the same manner on all protoplasm.

## Literature.

**Budington, R. A.**

'19 The Influence of Certain Ductless Gland Substances on the Growth of Plant Tissues. Biol. Bull., 37, 3, 188–193.

**Gudernatsch, J. F.**

'12 Feeding Experiments on Tadpoles. I. Arch. f. Entwick., 35, 3, 457–483.

'13 Feeding Experiments on Tadpoles. II. Amer. Jour. Anat., 15, 4, 431–482.

'15 Feeding Experiments on Rats. III. Amer. Jour. Physiol., 36, 4, 370–379.

**Haberlandt, G.**

'14 Physiological Plant Anatomy, p. 90. Macmillan and Co.

**Kunkel, B. W.**

'18 The Effects of the Ductless Glands on the Development of the Flesh-flies. Jour. Exper. Zoöl., 26, 255–264.

**Smith, E. F.**

'20 Bacterial Diseases of Plants (Crown Gall, p. 413–472). W. B. Saunders and Co.